# The Contractile Fine Structure of Vertebrate Smooth Muscle

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## I. THE IDENTITY OF THE CONTRACTILE MECHANISM IN SMOOTH AND STRIATED MUSCLES

About 30 years ago, Ernst Fischer introduced a new approach to muscle research by comparing the fine structure, and the function of the contractile mechanism of smooth and striated muscle. At that time (Fischer, 1936a and b; 1938) he systematically and successfully investigated the total, the intrinsic, and the form birefringence of smooth muscles and compared his results with analogous data concerning the contractile structure (Noll and Weber, 1935) and the oriented actomyosin threads (Weber, 1935) of skeletal muscle. These investigations were especially important because the birefringence of all muscles is based on its contractile structure and functional state, and because birefringence was better understood in micellar and molecular terms* since Wiener's theory.

It was found that the total birefringence of all smooth muscles (Fischer, 1936a and b; 1938) and skeletal muscles (Noll and Weber, 1935; Weber, 1935) is composed of intrinsic and form birefringence, and that the diffraction coefficient in both striated and smooth muscle deviates little from the value of 1.5. On the other hand, Fischer (1935a and b; 1938) found considerable quantitative differences in the amount of intrinsic and form birefringence, even in the case of different smooth muscles. The differences between smooth and striated muscle appeared especially large when the volume fraction of the birefringent elements was calculated from the form birefringence. The volume fraction of the double birefringent portion appeared to be about 10 times smaller in the smooth muscles investigated than in striated muscle (Fischer, 1938). Consequently, the intrinsic birefringence of the *small* birefringent volume in smooth muscle ought to be about 10 times larger than that of the *large* volume involved in striated muscle. This follows from the fact that the birefringence of the entire muscle-volume is similar in both types of muscle. This raises some problems! On the other hand, from the qualitative similarity in the birefringence of smooth and striated muscle, Fischer was able to conclude that the contractile fine structure of smooth and striated muscle was essentially identical (1936a and b; 1938).

In the meantime, additional important evidence was obtained with chemical and other (non-morphological) methods which strongly suggested that the contractile mechanism in all kinds of muscle and even in contractile cells such as fibroblasts, amebae, and thrombocytes was identical. This consisted of five main points:

1) From all kinds of muscle, including smooth muscle (Naeslund and Snellman, 1951) and contractile cells (Hoffman-Berling 1956; 1958), a typical contractile protein can be extracted which consists of two components, myosin and F-actin (*see also* table 1).

2) The ratio of the two components is about the same in smooth and striated muscle, i.e., about 3 or 4 g myosin per one gram actin (*see* table 1).

3) Most importantly, the contractile structures of all kinds of muscle (Bohr, Filo, and Guthe, 1962; Hasselbach and Ledermair, 1958; Huxley, 1963; Weber, 1958; Ulbrecht and Ulbrecht, 1952) and contractile cells (Hoffman-Berling, 1956) contract and relax under identical conditions when they are functionally isolated by extraction of the soluble muscle components. Contraction and relaxation occur only in the presence of ATP when $Mg^{++}$ ions are also present (e.g. in smooth muscle, Hasselbach and Ledermair, 1958). Contraction is always maximal when the concentration of free calcium ions is about $10^{-5}$ M, and relaxation is complete when the free calcium concentration is below $10^{-7}$ M (fig. 1; *see also* Hasselbach, 1964).

4) The ATPase activity is high during contraction and low during relaxation and rest (cf. figs. 1 and 2).

5) In cross-striated muscle (Hasselbach, 1964; 1960; Barany and Jaisle, 1960) and probably also in smooth muscle, this behavior of the ATPase activity depends on the association of myosin and actin

* In the 1930's, the x-ray diffraction patterns of muscle (Astbury and Dickinson, 1935; Boehm, 1931; Boehm and Weber, 1932) were unspecific because of their technical imperfection and because only wide angle diffraction patterns were possible. It is therefore not astonishing that these patterns appeared to be identical, not only in all types of muscle, but also in other fibrous protein structures such as keratin and fibrin.

during contraction, and on their dissociation during relaxation.

This physicochemical and functional similarity in the contractile complex of smooth and striated muscles has its counterpart in the similarity of its components—actin and myosin. The myosin component contains ATPase activity which is activated by Ca ions and inhibited by Mg ions in striated muscles (Barany and Jaisle, 1960) or at least not activated by Mg ions in smooth muscles (Gaspard-Godfroid, 1964; Needham and Cawkwell, 1956; Schirmer, 1965). However, the intact F-actin component has no ATPase activity in smooth muscles (Rüegg, Strassner, and Schirmer, 1965). All kinds of myosin have a similar sedimentation constant of about 6 S (in smooth muscles, Cohen, Lowey, and Kucera, 1961; Laszt and Hamoir, 1961). The myosin component of all kinds of muscle is soluble even in the absence of ATP if the ionic strength is larger than 0.3 $\mu$, and barely soluble if it is smaller than 0.15 $\mu$. The purified myosins of smooth (Hanson and Lowy, 1963) and striated muscle (Huxley, 1963) precipitate at ionic strengths below 0.3 $\mu$, not as amorphous aggregates, but as "typical" myosin filaments with a polar structure.

The fibrous form of the actin component (F-actin) of smooth as well as striated muscle is transformed into the globular actin monomer (G-actin) in salt-free solutions. G-actin polymerizes again to form F-actin when salt and $Mg^{++}$ or $Ca^{++}$ ions are added (e.g. in smooth muscle, Carsten, 1965; Schirmer, 1965 table 2). The actin components of smooth and striated muscle are completely soluble in solutions of high and low ionic strength, in the G state as well as in the F state (e.g. in smooth muscle Hasselbach and Schneider, 1951). F-actin filaments from all types of muscle, including vertebrate smooth muscle (Hanson and Lowy, 1963; Shoenberg et al., in press) have a double helical structure of about 50 Å thickness (table 2).

The conditions for the formation of the actomyosin complex from actin and myosin of smooth and striated muscle are identical:

1) In the absence of ATP or other nucleosidetriphosphates, actin and myosin combine to form actomyosin quite independently of the ionic strength in the range of 0.1 to 0.6 $\mu$ (e.g. in smooth muscle Bohr et al., 1962; Naeslund and Snellman, 1951; Needham and Cawkwell, 1956).

2) In the presence of ATP the complex is dissociated into its components if the ionic strength is greater than 0.3 $\mu$ (e.g. in smooth muscle Bohr et al., 1962; Naeslund and Snellman, 1951; Needham and Cawkwell, 1956).

3) The formation of the actomyosin complex has the same functional consequences in all types of muscle. If ATP, $Mg^{++}$ ions and traces of $Ca^{++}$ are present, the actomyosin structure contracts and develops tension (fig. 2).

The contractile structures of smooth muscle (Hasselbach and Ledermair, 1958; Ulbrecht and Ulbrecht, 1952; Filo, Bohr, and Rüegg, 1965) and of striated muscle (Barany and Jaisle, 1960; Hasselbach, 1960; 1964; Weber, 1958) relax again *only* in the presence of ATP if the splitting of ATP is inhibited by a lack of $Ca^{++}$ ions, by interaction inhibitors or by SH-

TABLE 1
Actomyosin Content of Smooth and Striated Muscle

| Muscle | Actomyosin content in % of protein | Actin-Myosin Ratio | Author |
|---|---|---|---|
| Cross striated (rabbit) | 52 | 1 : 3 | Hasselbach and Schneider (1951) |
| Arteries (cow) | 3 | | Rüegg et al. (1965) |
| Uterus (rabbit) | 2 to 4 | 1 : 4 | Needham and Williams (1963) |
| Taenia coli | 3 | | Rüegg et al. (1965) |
| Byssus retractor (clam) | 25 | | Rüegg (1961) |

TABLE 2
Double Helices and Reversible Depolymerization of Actin in Smooth and Striated Muscle

| Muscle | Actin filaments with double helical structure according to: (author) | Reversible polymerization and depolymerization according to: (author) |
|---|---|---|
| Cross striated (rabbit) | Hanson and Lowy (1963) | Straub (1943) |
| Cross striated (clam) | Hanson and Lowy (1963) | |
| Cross striated (crab) | Peterson (1963) | |
| Uterus (rabbit) | Hanson and Lowy (1963) | Carsten (1965) |
| Taenia coli | Hanson and Lowy (1963) | |
| Arteries (cow) | Shoenberg et al., in press | Rüegg et al. (1965) |
| Smooth adductor (clam) | Hanson and Lowy (1963) | |

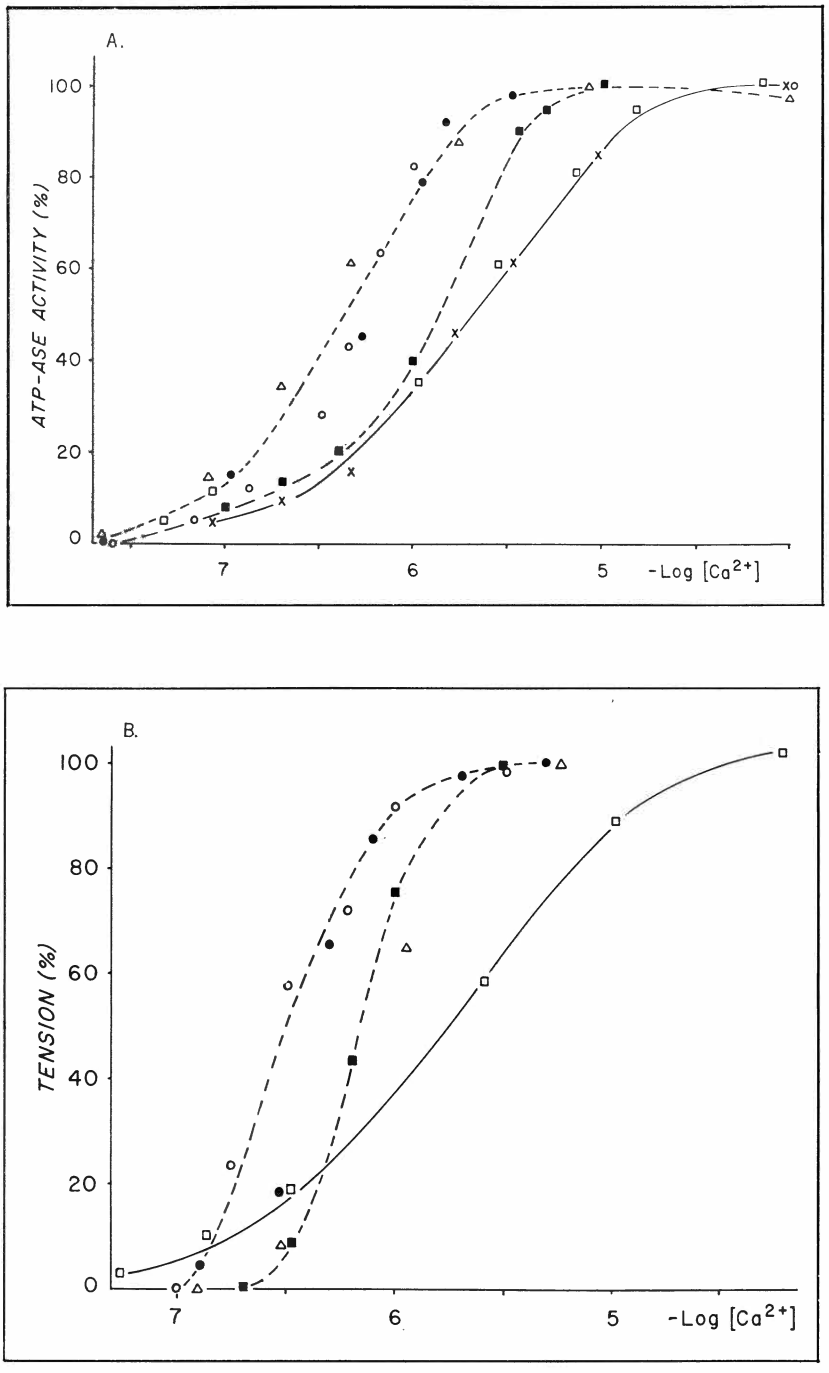


Fig. 1—The calcium requirement for the activation of smooth and cross striated glycerol extracted muscle fibers or myofibrils. Abscissa: negative logarithm of the free calcium ion concentration. Ordinate: *A,* ATPase activity; *B,* tension, both as percent of maximal activation; 0 = activity in the absence of calcium activation. Cross striated muscle is represented by the broken line, smooth muscle by the solid line. □ taenia coli of the guinea pig, △ heart of the dog, ● leg muscle of insect (Schaedler, in preparation); ■ leg muscle of the crab (Portzehl et al., 1965); X byssus retractor of the clam (Schaedler, in preparation); ○ skeletal muscle of the rabbit (*A,* Weber et al., 1964; *B,* Filo et al., 1965). Composition of solution: Mg-ATP 5 mM; Ca EGTA buffer 4mM; KCl 0.05; histidine buffer 0.02 to 0.05 M, pH 7; temperature 20 C.

reagents. The actin and myosin components from different muscles are so similar that actin and myosin from smooth and striated muscle (Schirmer, 1965) and even from thrombocytes and striated muscle (Bettex-Galland and Lüscher, 1965) may be recombined to form hybrid contractile actomysin complexes.

The functional identity of smooth and striated muscle provides strong evidence in favor of the conclusion which Fischer derived from his comparison of the double refringence of the two types of muscle, i.e. the contractile structure seems to be practically identical.

The functional identity of smooth and striated muscle may, in addition, offer an explanation for Fischer's birefringence results. From studies with skeletal muscle we know that the contribution of the (double helical) actin filaments to the birefringence of muscle is very small. The birefringence of the I-bands which contain actin but no myosin is about 10 times smaller than the birefringence of the myosin containing A-bands. Consequently we may consider total birefringence as the sum of form birefringence due to the parallel arrangement of whole myosin filaments and of intrinsic birefringence due to the parallel arrangement of myosin molecules within each filament.

## II. DIFFERENCES IN STRUCTURE AND FUNCTIONAL PERFORMANCE OF ACTOMYOSINS FROM SMOOTH AND STRIATED MUSCLE

The relatively simple and uniform concept outlined in part I does not harmonize with the results of modern electronmicroscopy. The electronmicrographs of thin sections from paramyosin-free vertebrate smooth muscles show only actin filaments and no myosin filaments (Needham and Shoenberg, 1964; Hanson and Lowy,

1963; Elliott, 1964), in contrast to the electronmicrographs of striated muscles. The discrepancy between the presence of form birefringence and the absence of myosin filaments in smooth muscles may be based on the presence of birefringent paramyosin filaments in the retractor muscles of Phascolosoma and Thyone, investigated by Fischer. At least one of these muscles—retractor of Phascolosoma—gives a strong paramyosin x-ray diffraction pattern (Bear, 1945).

The lack of demonstrable myosin filaments in electronmicrographs of smooth muscle makes it difficult to apply the generally accepted concept of sliding filaments to the contractile mechanism of vertebrate smooth muscles. However, the details of contraction and chemistry (*see* part I) strongly suggest the presence of a similar contractile mechanism in all types of muscles and even in contractile cells. Consequently, information concerning the properties of myosin from smooth muscles is needed.

The filaments of all types of myosin are formed spontaneously as soon as the myosin becomes insoluble. If the ionic strength of ATP-free myosin solutions is lowered, the myosin does not form an amorphous precipitate but produces typical filaments of somewhat varying size, as outlined above (Huxley, 1963; Hanson and Lowy, 1963). It is therefore tempting to assume that under physiological conditions in the presence of ATP, smooth muscle myosin is soluble even at low ionic strength! In fact, the actomyosin of all investigated vertebrate smooth muscles is so soluble that it can be easily and completely extracted at 0.1 $\mu$.* Skeletal muscle actomyosin, on the other hand, is completely extracted only at ionic strengths above 0.3 $\mu$.

This unusually high extractability of smooth muscles is dependent on the presence of ATP from the extracted muscle. Unlike actomyosin from striated muscle, smooth muscle actomyosin is not only dissociated by ATP, but also the myosin component is rendered soluble by ATP even at low ionic strengths (Laszt and Hamoir, 1961; Schirmer, 1965). The dissociation of actomyosin and the solubility of the myosin component from smooth muscle can be recognized in ultracentrifugation studies, i.e. after sedimentation at 100,000 $\times$ $g$ the pellet contains actin almost free of ATPase activity.

The myosin in the supernatant is not completely dispersed. Its aggregation is shown by a sedimentation constant of 12 S (Laszt and Hamoir, 1961; Schirmer, 1965) as compared with the normal value of 6 S. These aggregates are so much smaller than the myosin filaments of striated muscle that, unlike these filaments, they cannot be seen in the electronmicroscope when they are negatively stained (Hanson and Lowy, 1963; Shoenberg et al., in press). The high dispersion and solubility of smooth muscle myosin is due to the presence of ATP. This is shown by the precipitation of smooth muscle actomyosin or myosin at low ionic strengths (*see also* part I) when ATP is removed by dialysis (Laszt and Hamoir, 1961). Actomyosin and myosin become soluble again after addition of ATP (Laszt and Hamoir, 1961).

Consequently, the actomyosin of smooth muscle may be purified by repeated cycles of solution and precipitation by ATP addition and removal (Rüegg et al., 1965). When such purification cycles are continued for three days the actomyosin is still as soluble as in the extract (Schirmer, 1965). However, after aging for about one week, even without purification, the solubility at low ionic strengths is greatly diminished (Schirmer, 1965). The high solubility of the native myosin component under physiological conditions satisfactorily accounts for lack of myosin filaments in smooth muscle, in spite of the fact that purified smooth muscle myosin aggregates spontaneously into filaments when the ionic strength is lowered in the absence of ATP (*see* part I). However, the ability of living smooth muscles to contract in spite of the absence of myosin filaments remains puzzling, since ATP-free actomyosin gels prepared from vertebrate smooth muscles do not contract after addition of ATP but dissolve (Laszt and Hamoir, 1961; Schirmer, 1965), in contrast to actomyosin from striated muscle which superprecipitates under these conditions.

This difference between contractile living smooth muscle and noncontractile isolated systems disappears under conditions in which the high solubility of the myosin component is abolished.

Thus the actomysin system isolated from vertebrate smooth muscles contracts:

1) if the actomyosin preparation is aged for about one week,

2) if the contractile system is functionally isolated by extraction of the fiber with 50% glycerol for several days (Schirmer, 1965),

3) if the actomyosin preparation is kept at pH 6 before ATP addition (Schirmer, 1965),

4) if synthetic actomyosin is synthesized from purified actin and myosin,

5) if the actomyosin is reprecipitated several times in the presence of about 5 mM $Ca^{++}$ ions (Filo, Bohr, and Rüegg, 1963). The high

* Since actomyosin prepared from smooth muscle can be extracted at 0.1 $\mu$ ionic strength as well as at 0.6 $\mu$ ionic strength, it was believed that there were two types of actomyosin (Laszt and Hamoir, 1961), one type dissolving as true actomyosin at 0.6 $\mu$ ionic strength, and another type, the so-called tonoactomyosin, dissolving at 0.1 $\mu$ ionic strength. However, it was found later (Rüegg et al., 1965) that the so-called tonoactomyosin is identical with the actomyosin extracted at high ionic strength and that no actomyosin can be extracted if the previous extraction of tonoactomyosin was exhaustive (Rüegg et al., 1965).

solubility disappears irreversibly with these treatments except in condition (3). If the pH of the living muscle is lowered to 6 in the presence of $CO_2$, the actomyosin is no longer extractable with pH 6 buffered solutions of low ionic strength. Subsequently it can still be extracted if the pH is readjusted to 7 (Schirmer, 1965).

The mechanism of contraction of isolated systems under all these conditions is almost certainly similar to the contraction of isolated actomyosin from skeletal muscle. Contraction is even possible in artificial actomyosin prepared by combining smooth muscle myosin with striated muscle actin and vice versa (Schirmer, 1965).

One wonders whether or not the high solubility of myosin and actomyosin in fresh actomyosin preparations is an artifact. Isolated actomyosin systems of smooth muscle are able to contract as soon as the high solubility of actomyosin and myosin is diminished, while within the living smooth muscle cell the actomyosin system is always able to contract. The possibility of an artifact must be seriously taken into account since a number of protein factors were isolated recently from skeletal muscles, including "native tropomyosin" (Ebashi, Ebashi, and Maruyama, 1964) and "inhibitor" (Perry, in press), both of which inhibit the interaction between actin and myosin and may also raise the solubility of myosin. Thus it seems possible (Filo et al, 1963) that smooth muscles contain similar solubilizing proteins which, however, may be located differently from actomyosin in situ so that they do not react with the contractile proteins in living muscle. After extraction these proteins would then be able to combine and react with actomyosin.

This possibility has so far not been confirmed experimentally. At least it is clear that the high actomyosin solubility in the ATP-containing extract is not caused by "native tropomyosin." The action of this factor is inhibited with about $10^{-6}$ M calcium ions (Ebashi et al., 1964) while the actomyosin in fresh extracts remains dissociated and soluble even after raising the $Ca^{++}$ ion calcium concentration to about $10^{-6}$ M with calcium EGTA [ethyl-eneglycol bis (amino-ethyl-ether)-N, N'-tetraacetic acid] buffers. There is no evidence that another of the actomyosin contaminating proteins is the "solubilizing factor." Actomyosin reprecipitated several times by the described "ATP method" still dissolves after addition of ATP, although most of the contaminating proteins, including free tropomyosin B, are removed by this method (Schirmer, 1965). If myosin is then separated from such actomyosin in the preparative ultracentrifuge, the high solubility of myosin at low ionic strengths is diminished. However, it must be remembered that this isolation procedure requires about a week which is sufficient time to make smooth muscle actomyosin ATP-insoluble and contractile through aging even without purification (Schirmer, 1965).

Since the other actomyosin treatments mentioned above under conditions 1), 2), 4), and 5) also involve about one week's aging, two alternatives remain:

1) Aging for about one week abolishes the high solubility of actomyosin through selective denaturation which does not impair contractile function and ATPase activity.

2) Within one week a solubilizing factor is removed by either purification or denaturation of the factor.

Even if the first of these possibilities pertains, native myosin would not be totally disolved in the *living* cell. In the presence of actin the solubility of fresh myosin under physiological conditions is about 3 to 4 mg per ml (Schirmer, 1965). From the actomyosin content of smooth muscle—5 mg in 1 ml muscle (Rüegg et al., 1965)—an intracellular myosin concentration of about 20 mg* per ml of muscle cells can be calculated. Thus it would appear that about 80% of the myosin exists as a gel structure and not as a sol. The question is why this structure ("insoluble" myosin) does not form the type of myosin filaments which are formed in vitro and which are so typical for striated muscle. It remains for future research to find out the detailed structure of smooth muscle myosin within the smooth muscle cell. It is very likely that the measurement of intrinsic and form birefringence applied so successfully by Ernst Fischer will be extremely helpful in defining this structural analysis.

* This calculation is based on the assumption that the connective tissue is about 25% and the extracellular space about 40% to 50% of the muscle volume, i.e. the intracellular space containing all the myosin is about four times smaller than the volume of the whole tissue.

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